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Primary research

Behavioral and antioxidant activity of a tosylbenz[g]indolamine derivative. A proposed better profile for a potential antipsychotic agent

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Abstract

Background: Tardive dyskinesia (TD) is a major limitation of older antipsychotics. Newer antipsychotics have various other side effects such as weight gain, hyperglycemia, etc. In a previous study we have shown that an indolamine molecule expresses a moderate binding affinity at the dopamine D_2 and serotonin 5-HT_{1A} receptors in in vitro competition binding assays. In the present work, we tested its p-toluenesulfonyl derivative (TPBIA) for behavioral effects in rats, related to interactions with central dopamine receptors and its antioxidant activity.

Methods: Adult male Fischer-344 rats grouped as: i) Untreated rats: TPBIA was administered i.p. in various doses ii) Apomorphine-treated rats: were treated with apomorphine (1 mg kg^{-1}, i.p.) 10 min after the administration of TPBIA. Afterwards the rats were placed individually in the activity cage and their motor behaviour was recorded for the next 30 min The antioxidant potential of TPBIA was investigated in the model of in vitro non enzymatic lipid peroxidation.

Results: i) In non-pretreated rats, TPBIA reduces the activity by 39 and 82% respectively, ii) In apomorphine pretreated rats, TPBIA reverses the hyperactivity and stereotype behaviour induced by apomorphine. Also TPBIA completely inhibits the peroxidation of rat liver microsome preparations at concentrations of 0.5, 0.25 and 0.1 mM.

Conclusion: TPBIA exerts dopamine antagonistic activity in the central nervous system. In addition, its antioxidant effect is a desirable property, since TD has been partially attributed, to oxidative stress. Further research is needed to test whether TPBIA may be used as an antipsychotic agent.

Background

It is well established that compounds which interact with central dopamine receptors have therapeutic potential in the treatment of conditions like Parkinson's disease and psychotic disorders. For the later treatment, it is known that tardive dyskinesia (TD) is a major limitation of chronic antipsychotic drug therapy at least with older (typical) antipsychotics.

There is increased awareness of the different ways in which this condition manifests itself and the variety of disabilities that TD produces. Although a substantial research has been stimulated to identify the underlying pathophysiological mechanisms of TD, they remain largely elusive. There are several hypotheses about the pathophysiology of TD (dopamine hypersensitivity, neurotoxicity, GABA insufficiency, noradrenergic dysfunction, structural abnormalities)[1], however the true mechanism remains unknown.

The hypothesis of dopamine hypersensitivity proposes that the nigrostriatal dopamine system develops increased sensitivity to dopamine as a consequence of chronic dopamine receptor blockade induced by neuroleptic drugs. There is an increased incidence and prevalence of involuntary hyperkinetic dyskinesia in patients receiving dopamine antagonists in most [1-3] but not all reports [4,5]. Dopamine antagonists usually suppress TD, whereas dopamine agonists aggravate TD symptoms [6].

An alternate, though highly speculative hypothesis, is the proposal that TD is due to neurotoxic effects induced by free radical byproducts from catecholamine metabolism. The basal ganglia, by virtue of their high oxidative metabolism, are vulnerable to membrane lipid peroxidation as a result of the increased catecholamine turnover induced by neuroleptic drugs [7-9]. It is known that vitamin E (a-tocopherol) serves as a free radical scavenger, thus reducing the cytotoxic effects of free radicals. Clinical studies have produced conflicting data in this area. The impression gained from these studies was that while vitamin E is safe and well-tolerated, it confers only modest benefits. Some studies do not support the hypothesis that TD is mediated through free radical damage to neurons [8,10,11] while others support that vitamin E appears to be effective in reducing the severity of TD, especially in patients who are young and have recently developed TD [12,13].

Early neuroleptic agents showed great antipsychotic promise initially, however, the induction of extrapyramidal side effects associated with their use constituted a significant problem. Atypical antipsychotics possess a lower extrapyramidal side effects liability and show a better efficacy in the treatment of negative and depressive symptoms as well as cognitive disorders associated with schizophrenia. These features have been related to a higher affinity to serotonin receptors. However, they brought about various side effects such as weight gain, hyperglycemia, cholesterol level elevation, and QT interval prolongation [14].

A novel antipsychotic agent with a mechanism of action different from all currently marketed typical and atypical antipsychotics is aripiprazole. This quinoline derivative exerts potent partial agonistic action on D_2 and 5-HT_{1A} receptors and antagonistic properties at 5-HT_{2A} receptors. Aripiprazole claims to be the first agent of a third generation of antipsychotics, the so-called "dopamine-serotonin stabilizers"[14].

In a previous study [15] we have shown that 6,7,8,9-tetrahydro-N,N,-di-n-propyl-1*H*-benz [g]indole-7-amine (**PBIA**) (Figure 1) acts *in vivo* as a functional dopamine receptor partial agonist. It is known that a partial agonist at any dose level can not produce the same maximal biological response as a full agonist even though the partial agonist binds as tightly and as well to the receptor as the full agonist. In sum, a partial agonist has high affinity for its receptor, but low intrinsic activity. **PBIA** is a moderate [^{3}H]-spiperone and 8-OH-[^{3}H]-DPAT competitor. Spiperone is a selective D_2 antagonist while 8-OH-DPAT is a selective 5-HT_{1A} agonist. This means that **PBIA** expresses a moderate binding affinity at the dopamine D_2 and serotonin 5-HT_{1A} receptors in *in vitro* competition binding assays.

PBIA was designed as a metabolically stable bioisostere of the potent dopamine receptor agonist 5-OH-DPAT, (Figure 1). Phenolic dopamine receptor agonists suffer from poor bioavailability due to rapid metabolic inactivation via conjugation. Thus, an approach which has been pursued to overcome this problem is to develop non phenolic heterocyclic analogues. In this respect, evidence indicates that an indole NH moiety can be a bioisostere of the hydrogen-bonding H donor properties of the phenolic OH group in dopamine agonists. Based on the above, we synthesized **PBIA**.

In the present work, we tested the derivative **2** (Figure 1), 1-p-toluenesulfonyl-6,7,8,9-tetrhydro-N,N-di-n-propyl-

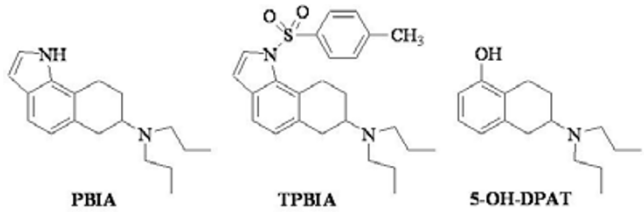

Figure 1
Structure of 6,7,8,9-tetrahydro-N,N,-di-n-propyl-1*H*-benz [g]indole-7-amine (PBIA), 1-p-toluenesulfonyl-6,7,8,9-tetrhydro-N,N-di-n-propyl-1*H*-benz [g]indol-7-amine (TPBIA) and 5-OH-DPAT

1*H*-benz [g]indol-7-amine (**TPBIA**) for behavioral effects in rats, related to interactions with central dopamine receptors. Because **TPBIA** has an increased lipophilicity and an appropriate polar molecular surface area (PSA) value, we hypothesized that it might be capable of penetrating the blood-brain barrier in a considerable degree. Additionally, the presence of the tosyl group might shift the agonistic activity to that of an antagonist. It is documented that increasing the van der Waals molecular volume of an agonist makes it an antagonist [16]. Finally, since free radical and oxidative stress may be implicated in the pathophysiology of a number of neurodegenerative diseases [17] we also investigated the antioxidant potential of **TPBIA**, since there are some reports concerning the role of free radicals in TD [7].

Therefore, it becomes interesting to design compounds that maintain antipsychotic efficacy and simultaneously could be free of TD risk.

The aim of the current study was:

1) to find if **TPBIA** crosses the blood-brain barrier,

2) to test the behavioral effects of **TPBIA** with specific focus on neuroleptic effects,

3) to test its antioxidant activity.

Materials and Methods

Synthesis of TPBIA

Key step of the synthesis was a Mukaiyama type aldol condensation between the dimethyl acetal of 1-(*p*-toluenesulfonyl)pyrrole-3-acetaldehyde and 4-di-*n*-propylamino-1-trimethylsilyloxycyclohexene followed by cycloaromatization under acidic conditions. A detailed description of the procedures can be found elsewhere [18]. TPBIA was isolated as its hydrochloride salt. It was a white crystalline solid with melting point of 209–211°C. The salt was soluble in water in contrast to its free base form.

Experimental Animals

Adult male Fischer-344 rats (~250 g) were used.

The experimental animals were grouped as:

i. *Group A: Untreated rats*: **TPBIA** was administered i.p. in various doses and immediately afterwards the rats were placed individually in the activity cage and their motor behavior was recorded for the next 30 min.

ii. *Group B: Apomorphine-treated rats*: the motor activity was measured as described above in the rats treated with apomorphine (1 mg kg^{-1}, i.p.) 10 min after the administration of TPBIA.

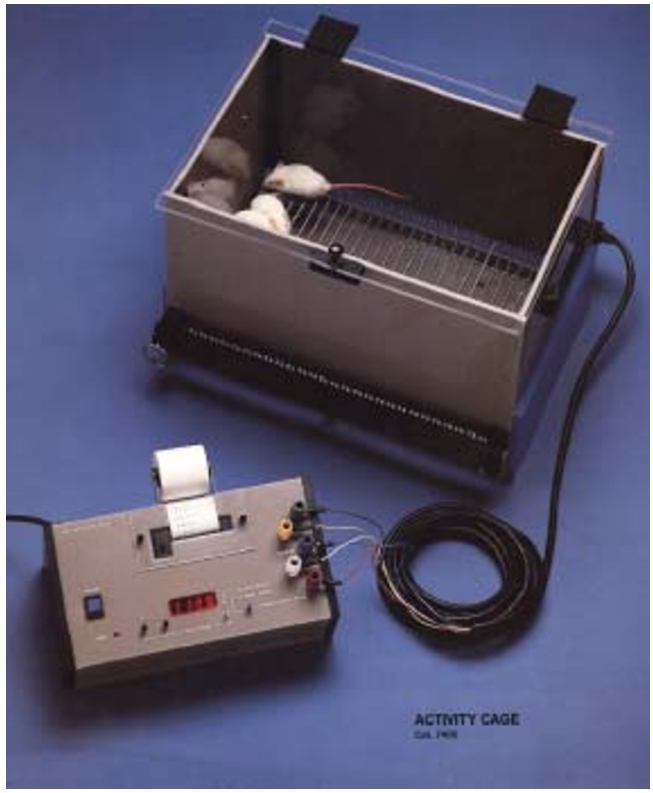

Figure 2
The Ugo-Basile activity cage (type 7401)

Biological Experimental Procedure

in vivo

The experiments were conducted according to a previous reported methodology [15]. **TPBIA** was converted to its hydrochloride salt and dissolved in water. Apomorphine was dissolved in 1 mM citric acid solution. The motor activity of the rats was measured between 12-6 pm in an Ugo-Basile activity cage (type 7401) (Figure 2).

in vitro

The antioxidant potential of **TPBIA** was investigated in the model of *in vitro* non enzymatic lipid peroxidation [19].

The experiments were conducted according to a previous reported methodology [15]. Hepatic microsomal fractions prepared from untreated male Fischer-344 rats were heat-inactivated (90°C, 90 s) and suspended in Tris-HCl/KCl buffer (50 mM/150 mM, pH 7.4). The incubation mixtures contained the microsomal fraction, corresponding to 0.125 g liver mL^{-1}, ascorbic acid (0.2 mM) in Tris buffer, and various concentrations (0.01–1 mM) of the tested compounds dissolved in DMSO. An equal volume of the solvent (0.1 mL) was added to the control incubate.

Table 1: Motor behavior of untreated rats

Compound (dose, μmol Kg^{-1})	Movements (±SEM) / 30 min	Compared with the control group (%)
Controls	263(81)	100
TPBIA(40)	161(22)^{NS}	61
TPBIA(80)	46(16)**	18

^{NS}, P > 0.05 (not significant) and **P < 0.01 according to Student's test, n = 3–6

Table 2: Motor behavior of apomorphine treated rats

Compound (dose, μmol Kg^{-1})	Movements (± SEM) / 30 min	Compared with the control group (%)
Apomorphine treated controls	385(68)	100
TPBIA(80)	113(28)**	29

**P < 0.01 according to Student's test, n = 4

The reaction was initiated by adding freshly prepared $FeSO_4$ solution (10 μM). The mixture was incubated at 37°C for 45 min. Aliquots (0.3 mL) of the incubation mixture (final volume 4 mL) were taken at various time intervals. Lipid peroxidation was assayed spectrophotometrically (535 nm against 600 nm) by determination of the 2-thiobarbituric acid reactive material.

Antioxidants inhibit the production of malondialdehyde and, therefore, the color produced after addition of 2-thiobarbituric acid is less intense. None of the compounds interfered with the assay, neither with the conjugation of 2-thiobarbituric acid or with the absorption at 535–600 nm. Each experiment was performed at least in duplicate. The UV measurements were carried out on a Perkin-Elmer 554 spectrophotometer.

Results

The effect of the TPBIA on the motor behavior of non treated and apomorphine pretreated rats are shown in Tables 1, 2 and Figure 3. Apomorphine is a selective agonist of the dopamine D2 receptors. It was found that:

i. In non-pretreated rats, **TPBIA** at doses of 40 and 80 μmol/kg reduces the activity by 39 and 82% respectively (Number of experimental animals: 3–6).

ii. In apomorphine pretreated rats, **TPBIA** (80 μmol/kg) reverses the hyperactivity and stereotype behavior induced by apomorphine (Number of experimental animals: 4).

The time course of non enzymatic lipid peroxidation as affected by 0.5, 0.25 and 0.1 mM concentrations of **TPBIA** is shown in Figure 4.

Discussion

The results support our hypothesis that:

a) TPBIA crosses the blood-brain barrier,

b) modifies the motor behavior of the experimental animals,

c) shows antioxidant activity.

a) The Polar Surface Area (PSA) of a molecule is defined as the area of its van der Waals surface that arises from oxygen and nitrogen atoms as well as hydrogen atoms attached to oxygen or nitrogen atoms. As such, it is clearly related to the capacity of a compound to form hydrogen bonds. PSA has been established as a valuable physicochemical parameter for the prediction of a number of properties related to the pharmacokinetic profile of drugs. Among these properties are the intestinal absorption and the blood-brain barrier penetration. PSA has been found to be useful in modeling intestinal absorption together with a direct estimate of lipophilicity widely acknowledged as an important factor in transport across membranes. A common measure of the degree of BBB penetration is the ratio of the steady-state concentrations of the drug molecule in the brain and in the blood, usually expressed as log(Cbrain/Cblood). We expect that the increased lipophilicity (calculated [20] ClogP = 6.659) and the small PSA value (calculated [21], 38.9 $Angstroems^2$) of this compound will facilitate its central

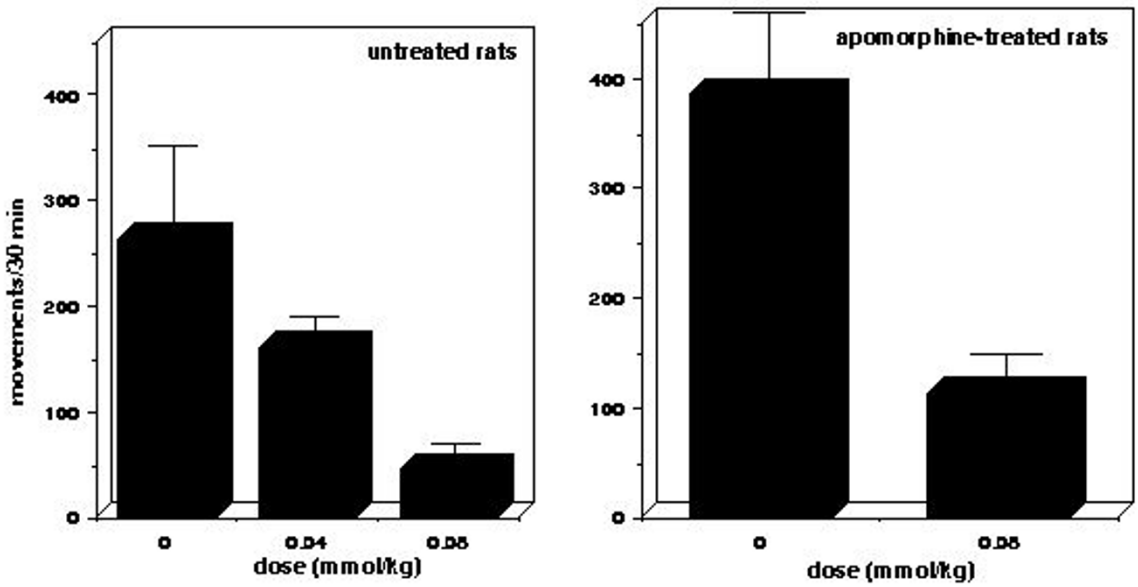

Figure 3
Effect of **TPBIA** on the motor behavior of experimental animals

nervous system penetration. Thus by using an equation reported by Clark et al [22] we found that the steady-state distribution of **TPBIA** between brain and blood is approximately 1000/1 (logBB = 0.58). This computational model contains two variables: PSA and calculated logP, both of which can be rapidly computed. The model could be considered reliable; for example the measured and predicted BBB permeability of the antidepressant drug, amitryptyline were quite similar (experimental logBB = 0.76–0.98 and calculated logBB = 0.76). Finally, its low PSA value is a strong indication that it could be used *per os* for systematic use [23].

b) The presented results could suggest that **TPBIA** acts as a dopamine receptor antagonist in the central nervous system. The tosyl group in **TPBIA**, which plain was found to be perpendicular to that of the indole ring in its low energy conformation (Figure 4) [24] is important to the differentiation of the biological profile between compounds **PBIA** and **TPBIA**. The association of increasing molecular weight with increasing antagonistic power is well known. An antagonist is always bulkier than the corresponding agonist and it is obvious that the likelihood of forming extra van der Waals bonds with the receptor increases the chances of the bulkier molecule having a longer retention time. Because a molecule's kinetic energy of translation (which is an important factor in desorption) does not change with increase in molecular weight, any gain in size by the molecule increases its time of residence on the receptor [16].

c) Some clinical studies [6,25] have shown that vitamin E (a well established antioxidant) may be effective in treating TD. However vitamin E does not cross readily the blood-brain barrier [26], which could explain why other studies failed to confirm these results [6]. Therefore we considered interesting to investigate the antioxidant potential of the synthesized **TPBIA**. It was found that **TPBIA** completely inhibits the peroxidation of rat liver microsome preparations at the studied concentrations

Conclusion

The results of the current study suggest that **TPBIA** crosses the blood-brain barrier, possesses neuroleptic activity and exerts antioxidative activity. The above constitute preliminary *in vivo/vitro* evidence suggesting that **TPBIA** could

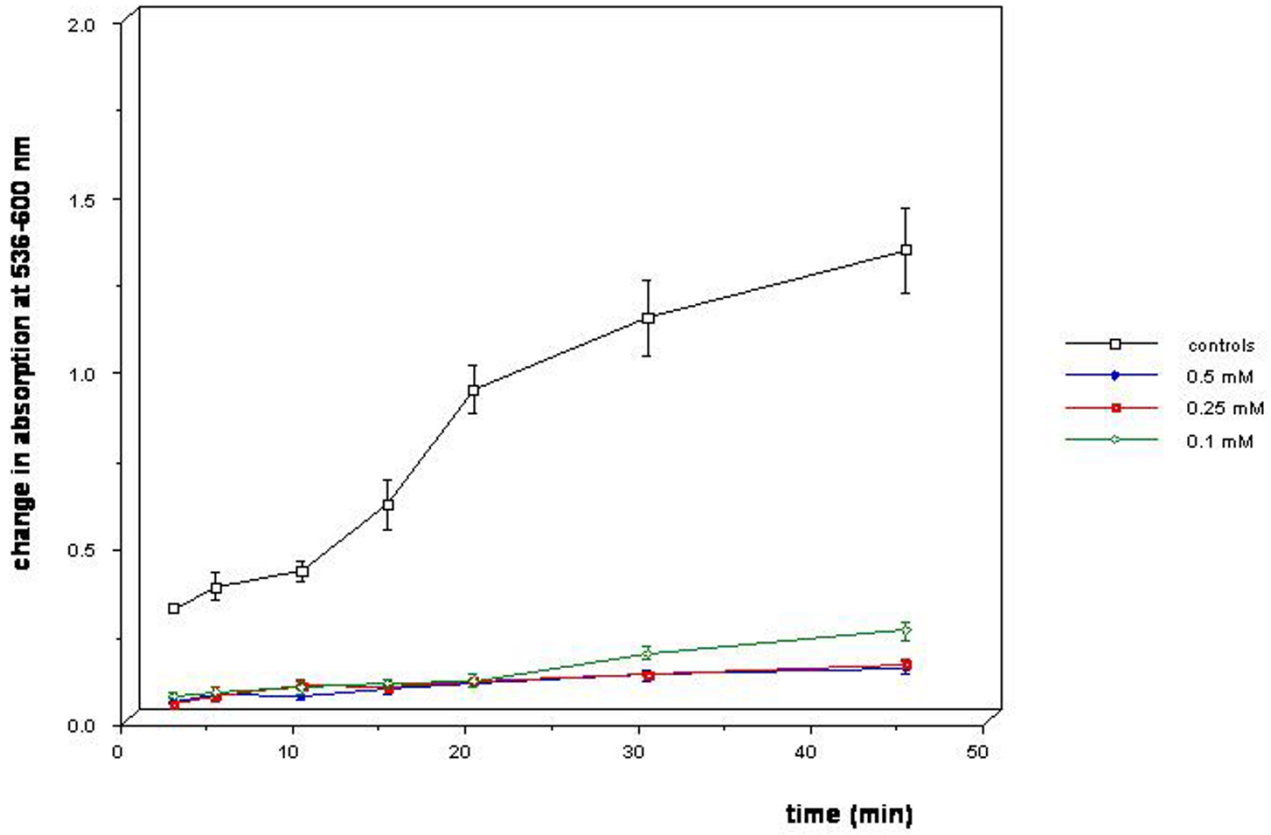

Figure 4
Time course of lipid peroxidation as affected by various concentrations of **TPBIA**.

merit further investigation as a potential candidate as an antipsychotic agent with novel and clinically important properties.

A putative combination of dopaminergic antagonism and antioxidant activity of a compound which readily cross the blood-brain barrier could be of pharmaceutical interest especially when the compound is used for the treatment of behavioral disorders in the frame of an organic or degenerative mental disorder.

The above results indicate that **TPBIA** might have therapeutic potential in the treatment of psychosis, due to its dopamine antagonistic activity in the central nervous system. In addition, its antioxidant effects is a desirable property, since tardive dyskinesia – a neuroleptics' severe side effect – has been attributed, at least in part, to oxidative stress.

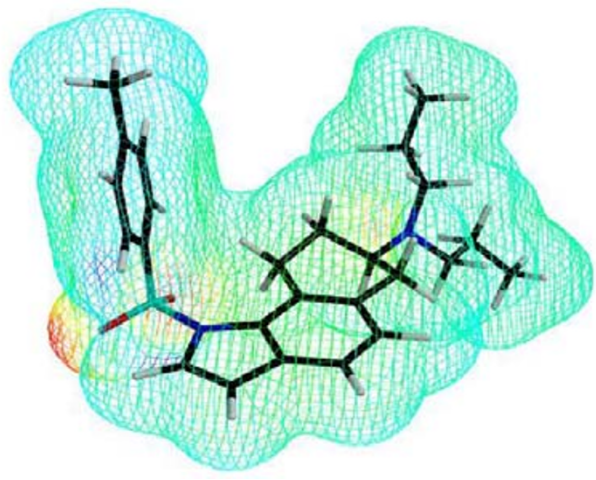

Figure 5
Low energy conformation and van der Waals surface of compound **TPBIA**

Conflict of interest

None declared.

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